

**DISSERTATION ON**  
**PREVELANCE OF ANTI TPO ANTIBODY IN INSULIN DEPENDENT**  
**DIABETES IN A TERTIARY CARE CENTRE.**



**SUBMITTED FOR**  
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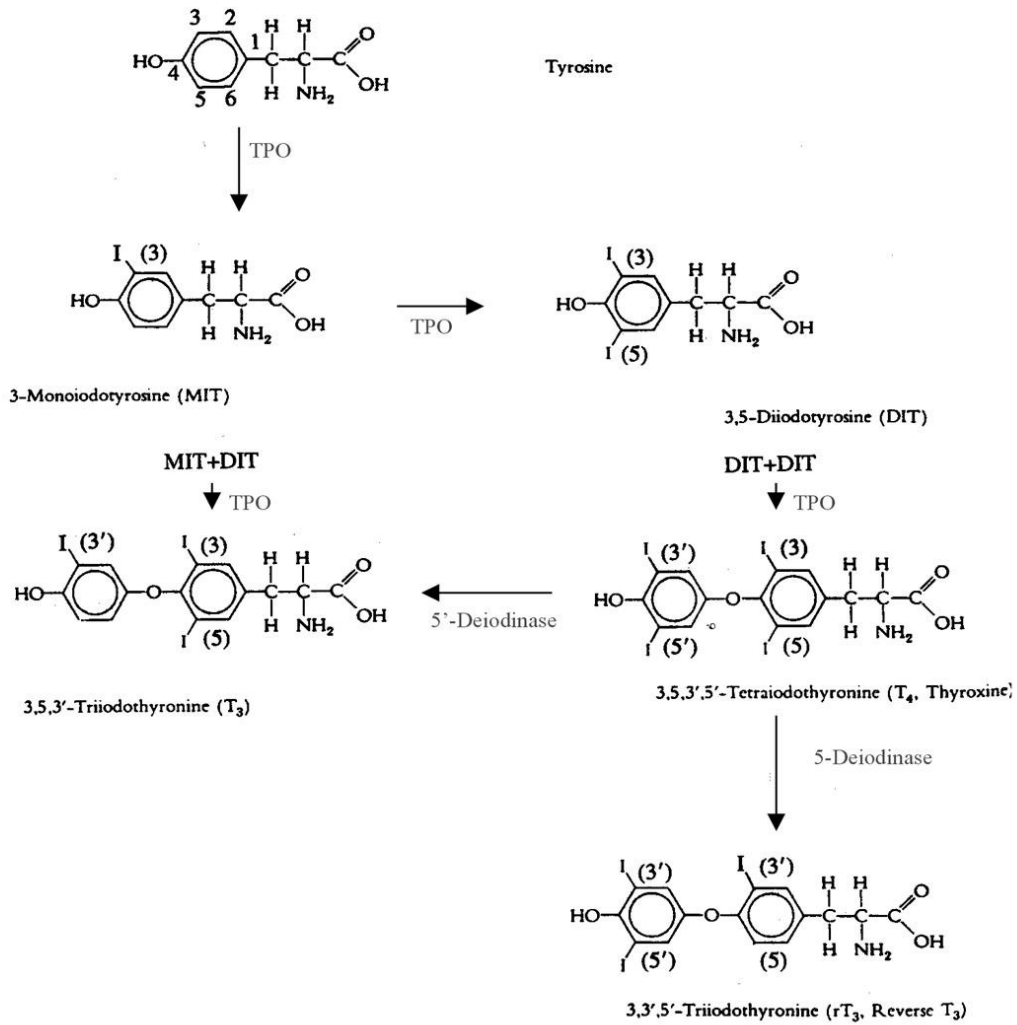
# DISSERTATION ON PREVELANCE OF ANTI TPO ANTIBODY IN INSULIN DEPENDENT DIABETES IN A TERTIARY CARE CENTRE

## INTRODUCTION

There are a number of auto antibodies associated with the auto immune thyroid diseases, which are characterized as either primary or secondary antibodies. Primary antibodies are directly pathogenic and often directed against cell membrane receptors, whilst secondary antibodies do not appear to be involved in pathogenesis but can serve as a useful diagnostic marker for the presence of autoimmune thyroid disease. Thyroid peroxidase (TPO) antibodies are one of the major secondary antibodies associated with autoimmune thyroid disease.

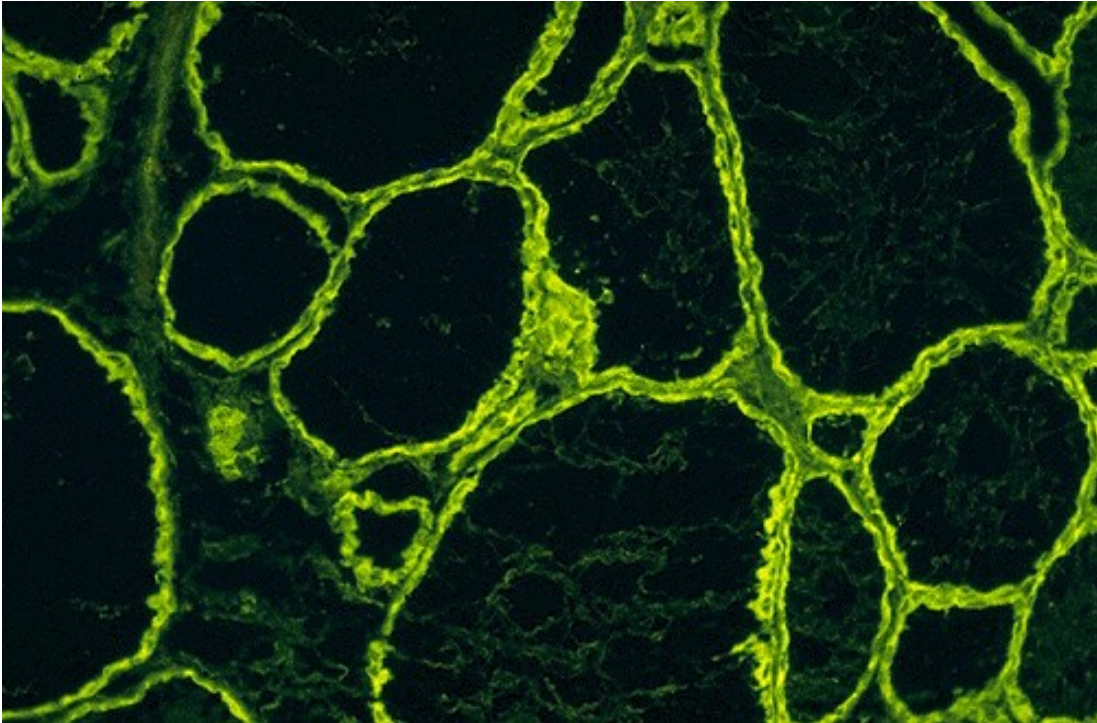
TPO was previously known as thyroid microsomal antigen. It is a 107 KD enzyme which is involved in thyroid hormone synthesis. TPO is located both on the cell surface and within the cytoplasm of thyroid acinar cells, bound to the vesicle which transports newly synthesized thyroglobulin, where it is involved in the iodination of thyroglobulin. High affinity antibodies (predominantly IgG) directed against TPO is found at elevated levels in the serum of patients with autoimmune thyroid disease such as Graves's disease, Hashimoto's thyroiditis, Diabetes mellitus and myxoedema. Autoimmune thyroid disease (AITD) causes cellular damage and alters thyroid gland function by humoral and cell-mediated mechanisms.

Cellular damage occurs when sensitized T-lymphocytes and/or auto antibodies bind to thyroid cell membranes causing cell lysis and inflammatory reactions. Alterations in thyroid gland function result from the action of stimulating or blocking auto antibodies on cell membrane receptors. Three principal thyroid auto antigens are involved in AITD. These are thyroperoxidase (TPO), thyroglobulin (Tg) and the TSH receptor. Other auto antigens, such as the Sodium Iodide Symporter (NIS) have also been described, but as yet have no diagnostic role in thyroid autoimmunity.

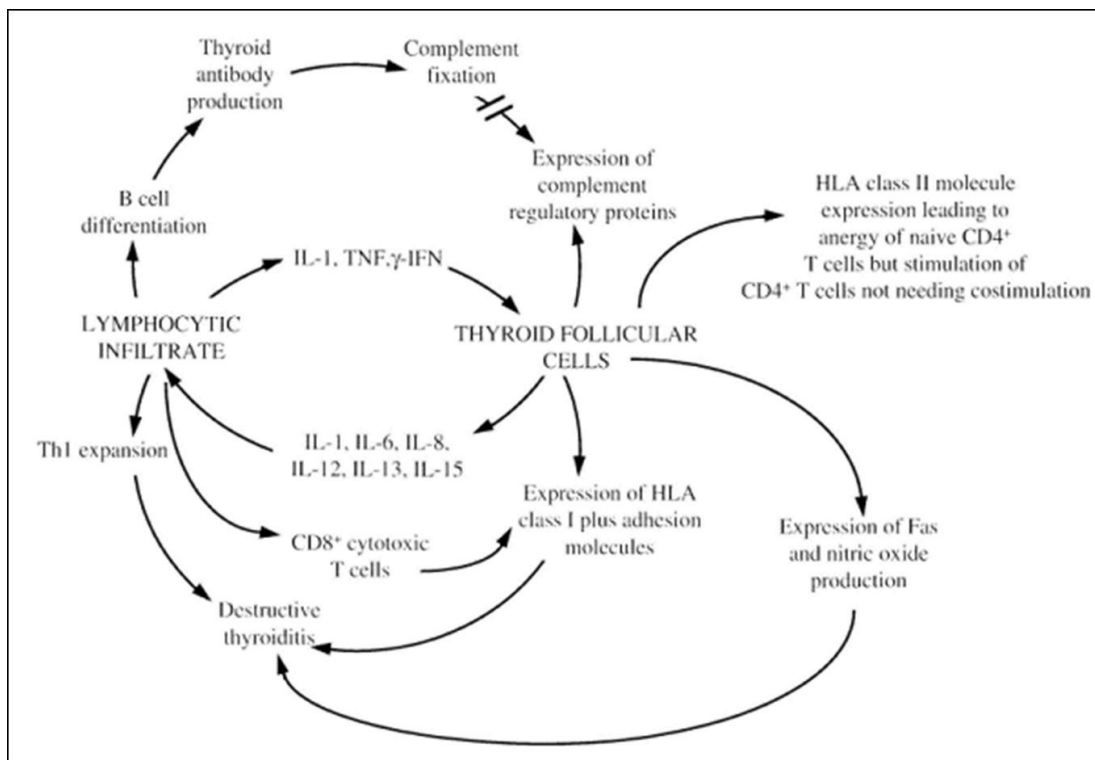


## CLINICAL SIGNIFICANCE OF THYROID AUTOANTIBODIES

TPO antibodies (TPOAb) appear to be involved in the tissue destructive processes associated with the hypothyroidism observed in Hashimoto's and atrophic thyroiditis. The appearance of TPOAb usually precedes the development of thyroid dysfunction. Some studies suggest that TPOAb may be cytotoxic to the thyroid. TPOAb and/or TgAb are frequently present in the sera of patients with AITD.



The prevalence of thyroid auto antibodies is increased when patients have non-thyroid autoimmune diseases such as type 1 diabetes and pernicious anemia. Ageing is also associated with the appearance of thyroid auto antibodies and increased prevalence of AITD.



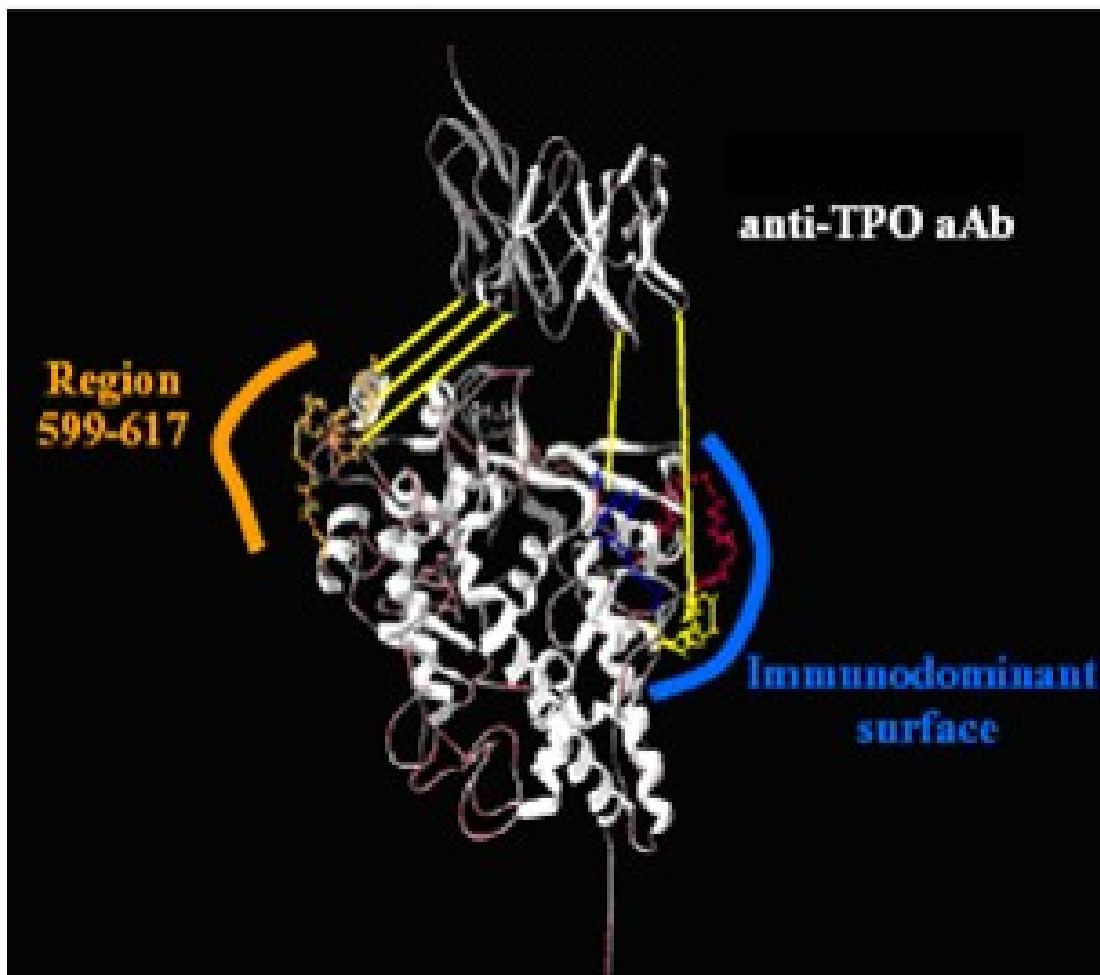
## THYROID DISEASE AND DIABETES:

Thyroid disease is widespread and prevalence increases with advancing age. However, as assessing thyroid function is reliable and inexpensive, certain high risk groups—such as neonates, the elderly and diabetics – should undergo regular screening.

Diabetes mellitus (DM) is a multisystem disease with both biochemical and anatomical consequences. It is a chronic disease of carbohydrate, fat, and protein metabolism caused by the lack of insulin. In type 1 diabetes, insulin is functionally absent because of the destruction of the beta cells of the pancreas. Type 1 DM occurs most commonly in juveniles but can occur in adults, especially in those in their late 30s and early 40s.

Diabetes mellitus is thought to be, in some cases, an auto-immune disease caused when antibodies attack certain cells of the pancreas, affecting the production of insulin. In patients or families where auto-immune thyroid disease exists, this type of diabetes mellitus may develop, sometimes in younger members of the family.

This should be suspected particularly if symptoms of tiredness and weight loss develop together with increased thirst and passing of large volumes of urine.



Clinically, thyroid dysfunction may undermine diabetes control. For example, hyperthyroidism may worsen glycemic control and increase insulin requirements. Indeed, thyrotoxicosis may unmask subclinical diabetes.

While hypothyroidism markedly alters carbohydrate metabolism, such changes are rarely clinically significant. However, as less insulin is degraded, the exogenous insulin requirement may be lower. Moreover, hypothyroidism often produces dyslipidaemias, including elevated triglyceride and low-density lipoprotein (LDL) cholesterol concentrations.



Therefore, hypothyroidism can exacerbate coexisting dyslipidaemias in type 2 diabetes. Thyroxine reverses these lipid abnormalities. That diagnosing thyroid dysfunction can be difficult. For example, poor glycaemic control produces symptoms similar to hyperthyroidism, such as weight loss despite increased appetite as well as fatigue. Clinicians need to be careful not to confuse severe diabetic nephropathy and hypothyroidism: both produce edema, fatigue, pallor and weight gains. Finally, poorly controlled diabetes may alter thyroid function.

Against this background, the serum TSH immunoassay offers the most reliable and sensitive screening test for thyroid dysfunction. However, screening for anti-thyroid peroxidase (TPO) antibodies in people with type 1 diabetes may predict autoimmune thyroid disorders.

Management is generally similar to that in the non-diabetic population. However, L-thyroxine therapy may exacerbate angina by increasing myocardial contractility and heart rate. Clinicians should consider treating subclinical hypothyroidism if patients either have elevated serum LDL cholesterol exacerbated by hypothyroidism or detectable serum anti-TPO antibodies.

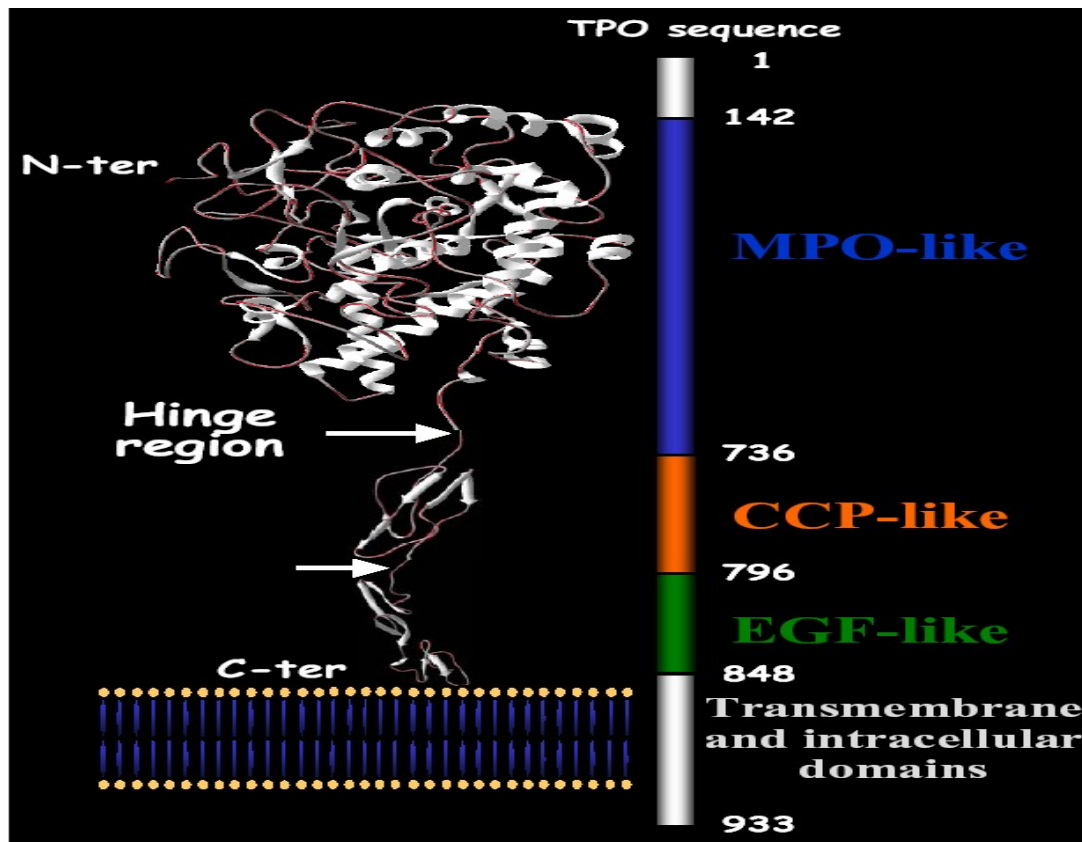
Thyroid dysfunction is common among diabetic patients and can produce metabolic disturbances. Therefore, regularly screening diabetic patients allows early treatment. Type 1 patients expressing anti-TPO antibodies should be screened annually. In anti-TPO negative patients, a TSH assay every two to three years suffices. Among patients suffering from type 2 diabetes, clinicians should consider a TSH at diagnosis and then at least every five years.

## **BIOCHEMISTRY OF THYROID PEROXIDASE (TPO)**

Thyroid Peroxidase (TPO) is a 110 KD membrane bound hemo-glycoprotein with a large extracellular domain, and a short transmembrane and intracellular domain. TPO is involved in thyroid hormone synthesis at the apical pole of the follicular cell. Several isoforms related to differential splicing of TPO RNA have been described. TPO molecules may also differ with respect to their three dimensional structure , extent of glycosylation and heme

binding. Most of the TPO molecules do not reach the apical membrane and are degraded intracellularly.

The human thyroid peroxidase (TPO) found in the thyroid follicular cells, is a high molecular weight glycoprotein containing hem prosthetic group. It plays a central role in the multi-step biosynthesis of thyroid hormones, T4 and T3.



Thyroid disorders are caused in most cases due to the production of auto-antibodies against different antigens of thyroid tissues. Most important auto-antibodies are those against thyroglobulin, thyroid peroxidase and the TSH receptor.

Anti-TPO is found in all thyroid autoimmune diseases, with the highest level observed in Hashimoto's thyroiditis. Elevated concentration of anti-TPO is also characteristic of idiopathic myxedema and chronic atrophic thyroiditis. Anti-TPO auto-antibodies are regarded as the indicator of developing thyroiditis during pregnancy, or in patients with familiar history of different autoimmune diseases (Type-1 diabetes mellitus, Addison's disease, pernicious anemia) .

## **OBJECTIVE OF THE STUDY**

1. To detect and measure TPO – Ab in young IDDM individuals.
2. To find out the Thyroid abnormalities in TPO – Ab positive individuals in IDDM – using Thyroid function tests.

## REVIEW OF LITERATURE

- 1) Hiroki Shimura et al auto immune endocrine diseases occur most frequently among various auto immune diseases. Type-1 DM & Grave's disease frequent in daily clinical practice. Auto immune mechanism are involved in many cases of Type-1DM Which ranging from rapid onset and slowly progresses over years.
- 2) Hiltunen during their study of immunization and Type- 1 diabetes mellitus found that there is no clear evidence of either to prevent or to induce diabetes in human.
- 3) PK.Moulik et al on their study of patients with diabetes who previously had thyroid function test found that 11% were TPO(+).
- 4) Guillermo and his colleagues during their cross-sectional studies in Type I diabetes confirms the association between auto immune thyroid disfunction and Type I diabetes. Patients who were TPO (+) 17.91 times likely to develop hypothyroidism. Their were no differences in BMI, Lipid profile between patients with (or) without thyroid disfunction. They conclude all subjects with Type I diabetes should undergo annual screening by TSH measurement to detect asymptomatic thyroid disfunction with (+) TPO antibody.
- 5) Kordonouri O et al in a multi-center survey found out that 21.6% elevated levels of anti TPO levels .Thyroid auto immunity more common in girls.
- 6) Prazny et al in their study on auto immunity in Type I DM with respect to diabetes control have found that auto antibodies in Type I diabetes patients could reveal sub-clinical cases of AITD. They have no influence on diabetic control. They suggest proper follow up of patients with positive auto antibodies.
- 7) H.G Bohnet found that use of cord blood is an alternative form to screen anti TPO.
- 8) A multi center survey conducted by Olga Kordonouri et al on Thyroid auto immunity in

children and adolescent with Type I diabetes found out 15.4% had raised anti TPO. Girls have more antibodies than boys. Measurement of anti TPO and TSH in yearly intervals after the age of twelve.

- 9) D.Hansen et al in a study on Thyroid function morphology and auto immunity in young patients with IDDM have found that there were no significance differences in Thyroid function variables.
- 10) A longitudinal study of first degree relations of Type I diabetes patients by E.Hatziagelakil et al found that thyroid auto immunity has to be tested regularly not only in diabetic but also in their first degree relatives.
- 11) I.H.De Leeuw done a cross-sectional study found that auto immune aggression is not limited to the pancreas but also to the other endocrine systems (Thyroid) were frequently found to be increased in Type I DM patients as compared non-diabetic populations.
- 12) Y.Vainilovich et al during their study over to adolescent with Type I DM with a duration of 5 – 15 years found that TPO AB increased in 13.7% and there is no correlation between duration of diabetes and TPO AB.
- 13) Immuno assay of anti thyroid auto antibodies by Renato Tozzoloi et al suggested that the use of high sensitive immuno metric methods in clinical laboratories to assay anti thyroid antibody has expanded.
- 14) Bilimoria and his colleagues on analyzes of Type I diabetes with auto immune thyroid disfunction found that TPO and TSH measurements are the most efficient cost effective combination of screening test.
- 15) D.Hansen et al on their prospective study of Thyroid function and auto immunity in young patients with Type I diabetes found that prevalence of TPO Ab at 13% and recommend screening using serum TSH in follow up of young diabetic patients.

- 16) A study on IDDM and auto immune thyroid disease in the Pediatric age group by Lorini et al found that screening for auto immunity even if the patients are asymptomatic. AITD is frequently associated with IDDM and the presence of Thyroid auto antibody has been considered as a risk factor for the development of hypo (or) hyper thyroidism.
- 17) K.F. Tait et al on their study found that females are more frequently affected by auto immune disease particularly AITD.
- 18) Natural course of auto immune thyroiditis in Type I diabetes: association with gender, age, diabetes duration and puberty – study conducted by O. Kardounouri et al found that among diabetes 17 had TPO AB, which may be used as a marker of Thyroid auto immunity.
- 19) Jin P et al in their study on adult onset latent auto immune diabetes and AITD found that 16% TPO Ab present in latent auto immune diabetes. The presence of Thyroid antibody may predict high risk of thyroid dysfunction.
- 20) J.C. Blair and J. Allgrove proposed a screening protocol for thyroid dysfunction co-exist with Type I diabetes thyroid function should be assessed annually in diabetic children. Thyroid auto antibodies are measured at diagnosis and repeated if TSH level raised.
- 21) A study done by Kalicka kasper czyk et al on TPO antibodies and thyroid diseases in children and adolescent with Type I diabetes mellitus found positive TPO Ab titer in 34.7% patients. 5% patients shows thyroid dysfunction.
- 22) De Louis da et al conducted a study on in Type I patients with their families concludes value of TPO Ab were higher in patients than families. No correlation between auto immunity and glycemic control.
- 23) In the diabetic volume study of the predictivity of thyroid auto antibodies in children and adolescents with Type I diabetes found that 10% of anti-TPO. Girls had more elevated anti-TPO. They conducted that 50% of children with diabetes and significant titres of anti-TPO – Ab.

- 24) Erin McCanlies et al during the study of Hashimoto's thyroiditis and insulin dependent diabetes mellitus suggested that gender risk factors may be primary determinants of Hashimoto's thyroiditis and other auto immune diseases among women.
- 25) Czerniawska E, Szalecki et al during the study on prevalence of thyroid antibodies TPO and ATGaE the onset of Type I diabetes mellitus in children found that auto immune Type I DM other organ specific antibodies particularly thyroid auto antibodies. TPO antibodies were 17.8% of children and in 19.2% of other centers.
- 26) Laurberg and his colleagues in the study on sensitive enzyme linked immunosorbent assay for measurement of auto antibodies to human thyroid peroxidase found that auto antibodies against TPO containing 70,7,07,0.02 and 0 u/ml-1. The new method is a valuable tool for studying the epidemiology of thyroid auto immunity.

## **MATERIALS AND METHODS**

Since the onset of insulin dependent diabetes which occurs in earlier years who were only on insulin therapy without any anti-thyroid (or) hormone (or) lipid lowering drugs. 60 persons were selected for this study.

This study was conducted at Tertiary Care Medical Centre, Thanjavur Medical College. All the participants were enquired by a questionnaire.

1.Name

2.Age

3.Address

4.Complaints

- Palpitation
- Lethargy
- Menstrual History in Female
- Past History
- Surgical History
- Cold Intolerance, Voice Change

Physical Examination Conducted

1.Pulse

2.Blood Pressure

3.Height

4.Weight

5.To rule out features suggesting any hypo (or) hyper thyroid Status.

Fasting blood specimen was collected from each participant plasma fasting glucose level were estimated GOD/POD method by auto analyzer.



Serum triiodo thyronine, serum thyroxine and thyroid stimulating hormone were estimated by enzyme immuno assay method.

Serum total cholesterol, serum triglycerides, serum low density lipo protein cholesterol and serum high density lipo protein cholesterol were estimated in the fasting serum sample.

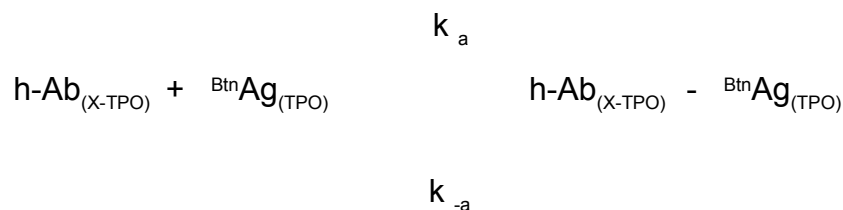
## ESTIMATION OF TPO ANTIBODIES BY ACCUBIND ELISA MICROWELLS

### PRINCIPLE

#### A SEQUENTIAL ELISA METHOD

The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenous added biotinylated thyroid peroxidase antigen.

Upon mixing biotinylated antigen and a serum containing the autoantibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the followed equation:



$\text{B}^{\text{tn}}\text{Ag}_{(\text{TPO})}$  = Biotinylated Antigen (Constant Quantity)

$\text{h-Ab}_{(\text{X-TPO})}$  = Human Auto-Antibody (Variable Quantity)

$$Ab_{(X-TPO)} - B^{tn}Ag_{(TPO)} = \text{Immune Complex (Variable Quantity)}$$

$k_a$  = Rate Constant of Association

$k_{-a}$  = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:



Streptavidin<sub>c.w</sub> = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and /or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.



$IC_{(h-IgG)}$  = Immobilized Immune Complex (Variable Quantity)

$\text{ENZ } Ab_{(X-h-IgG)}$  = Enzyme-antibody conjugate (Constant Quantity)

$\text{ENZ } Ab_{(X-h-IgG)} - IC_{(h-IgG)}$  = Antigen –Antibody Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted materials to the reacted materials which is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the

antibody activity of an unknown can be ascertained.

## **REAGENTS:**

### **A. Anti-TPO Calibrators – 1.0 ml/vial – Icons A-F**

Six (6) vials of references for anti-TPO at levels of 0(A),25(B),50(C),100(D),250(E) and 500(F) IU/ml. Store at 2-8°C. A preservative has been added.

### **B. TPO Biotin Conjugate -13ml/vial – Icon**

One (1) vial of biotinylated thyroid peroxidase antigen in a buffering matrix. A preservative has been added. Store at 2-8°C.

### **C. Enzyme-antigen Conjugate -13ml/vial – Icon**

One (1) vial of anti-human IgG-horseradish peroxidase (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8°C.

### **D. Streptavidin Coated Microplate -96 wells – Icon**

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2 - 8°C.

### **E. Serum Diluent Concentrate -- 20 ml**

One (1) vial of serum diluent containing buffer salts and a dye. Store at 2 - 8°C.

### **F. Wash Solution Concentrate -- 20ml – Icon**

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2 - 8°C.

### **G. Substrate A – 7ml/vial – Icon S<sup>A</sup>**

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2 - 8°C.

### **H. Substrate B – 7ml/vial – Icon S<sup>B</sup>**

One (1) bottle containing hydrogen peroxide(H<sub>2</sub>O<sub>2</sub>) in buffer. Store at 2 - 8°C.

- I. Stop Solution – 8ml/vial – Icon   
One (1) bottle containing a strong acid (1 N HCl). Store at 2 - 8°C.

## **REAGENT PREPARATION:**

1. Serum Diluent

Dilute the serum diluent concentrate to 200ml in a suitable container with distilled or deionized water. Store at 2 - 8° C.

2. Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27° C for up to 60 days.

3. Working Substrate Solution

Pour the contents of the vial labeled Solution 'A' into the vial labeled Solution 'B'. Mix and store at 2-8° C. Use within 60 days. Or for longer periods of usage determine the amount of reagent needed and prepare by mixing equal portions of Substrate A and Substrate B in a suitable container. For example, add 1ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made. Discard the unused portion).

Note: Do not use the working substrate if it looks blue.

4. Patient Sample Dilution (1/100)

Dispense 0.010ml (10µl) of each patient specimen into 1ml of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8° C for up to forty-eight (48) hours.

**TEST PROCEDURE:** Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27° C).

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or diluted patient specimen into the assigned well.
3. Add 0.100 ml (100µl) of TPO Biotinylated Conjugate Solution.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two(2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacture's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add 0.100 ml (100µl) of enzyme anti-h-IgG Conjugate Solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells.
9. Swirl the microplate gently, cover and incubate for thirty (30) minutes at room temperature.

10. Repeat steps (6&7) as explained above.
11. Add 0.100 ml (100µl) of Working Substrate Solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.
12. Incubate at room temperature for fifteen (15) minutes.
13. Add 0.050ml (50 µl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

Normal TPO – Ab level – Below Forty (40) IU / ml

## **ESTIMATION OF T3 BY ELISA METHOD**

### **PRINCIPLE**

In the Biotechx OptiCoat™ T3 EIA kit, antibody to T3 (produced in New Zealand white rabbits by administration of T3 that was coupled to a protein carrier), is the specific antibody immobilized on microwell plates. Purified T3 conjugated to the enzyme horseradish peroxidase (HRP), is used to detect T3. In order to accurately measure the Total T3 concentration in serum, the endogenous binding proteins (i.e., TBG, albumin and prealbumin ) are blocked by the use of 8-anilino-1-naphthalene sulfonic acid (ANS).

In the assay procedure, the T3 standard and/or patient serum is added along with T3-horseradish peroxidase conjugate to the antibody coated wells. A competition reaction results between the native antigen in the serum, and the enzyme-antigen conjugate for a limited number of solid phase binding sites. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen in a washing step by decantation or aspiration.

Enzyme substrate 3,3',5,5'-Tetramethylbenzidine (TMB) is then added to the microwell and incubated. In the presence of conjugate-antigen-antibody complex, TMB is hydrolyzed to a colored end product.

The enzyme activity in the antibody-bound fraction as measured by the intensity of the color development is inversely proportional to the native antigen concentration. The intensity of color is measured spectrophotometrically at 450 nm. The concentration of T3 is interpolated from a standard curve. The concentration of T3 in a given specimen, determined with assays from different manufactures, can vary due to differences in assay methods and reagent specification.

**1.MICROWELL PLATE: Cat.No.BL 4310,1 plate of 96 wells**

Microwell plates are coated with rabbit T3 antibody.Store bag with microwell plate at 2-8 °C. Allow bag to come to room temperature prior to opening. Once the bag is opened, the microwell strips should be resealed and stored in the bag with the dessicant. When stored in this manner the breakaway wells are stable until the date indicated on the kit.

**2.T3 CONJUGATE: Cat.No.BL 4320,10 mL**

Horseradish peroxidase conjugated to triiodothyronine in a buffered solution, containing bovine serum albumin, inhibitors, and preservatives.

**3.OneBlue™, TMB Single Substrate:Cat.No.BL 29932,22mL**

Contains 3,3',5,5'- Tetramethylbenzidine substrate reagent in a stabilized buffer system and urea hydrogen peroxide.

**4.STOP SOLUTION:Cat.No.BL 4250,12mL**

Hydrochloric acid, 1N

**5.STANDARDS:Cat.No.BL 3801-3806,0.5 mL/vial**

Six vials, T3 standards 0,50,100,250,500 and 1000 ng/dL, in T3 free human serum with thimerosal added as a preservative.

**6.CONTROL SERUM (Lyophilized):Cat.No.BL 2680**

Contains human serum and preservative. Reconstitute with 1mL of distilled water. Storage:Freeze at -15° C. Three tested cycles of freezing and thawing had no effect on T3 concentration.Stability: When stored, 1 year from date of reconstitution at -15° C

**7.WASH SOLUTION CONCENTRATE:Cat.No.BL 21-138, 15mL**

Buffer solution concentrate with thimerosal added as a preservative. Prepare working wash solution by adding the 15mL Wash Solution Concentrate to 500ml of distilled water. Store at 2-8° C. The working buffer is stable until the expiration of the kit.

**REAGENT PREPARATION:**



### **1. STANDARDS,CONTROL SERUM, AND PATIENT SPECIMEN.**

Ensure that the reagents are at room temperature prior to use.

### **2. SAMPLE PREPARATION,STORAGE, AND HANDLING**

Collect a minimum of 0.5mL of whole blood. Allow blood to clot, and separate the serum. Specimens can be stored for short-term (1day) at 2-8° C or for long-term at -20 Grossy hemolyzed, contaminated, lipemic,specimens and samples containing bilirubin should not be used. Avoid repeated freeze-thaw of samples. In the case of frozen samples, be sure to thaw samples completely before use.

### **3. STORAGE OF KIT**

The kit should be stored at 2-8° C. Do not freeze. Allow reagents to come to room temperature prior to use. Ensure that the microwell bag is sealed well with the dessicant. Failure to do so can result in deterioration of the antibody-coated wells.

## **TEST PROCEDURE**

Remove the appropriate number of microwell strips from the bag. Ensure that all unused strips remain in the bag, and that the bag is sealed well, and returned to the refrigerator. Include two wells each for the standards, control serum, and test sample(s).

1. Add 50 µL each of Standards, Control Serum, and Test sample(s) in the appropriate wells.
2. Add 2 drops or 100 µL of T3-Conjugate to each well. Complete additions within 5 minutes. Cover the plate. Mix wells by swirling the plate gently for 30 seconds and incubate at room temperature(22-25°C) for 1 hour.
3. Decant or aspirate the supernatant from all the wells. Drain thoroughly by inverting the plate and tapping vigorously on an absorbent paper towel to remove excess fluid.
4. Wash the wells five times with diluted wash solution. After each wash, completely

empty the wells as described above.

5. Add 4 drops or 200  $\mu\text{L}$  of OneBlue TMB-Substrate solution to each well. Mix wells by swirling the plate gently for 30 seconds. Complete all additions within 5 minutes. Cover the plate and incubate at room temperature (22-25°C) for 30 minutes.
6. Add 2 drops or 100  $\mu\text{L}$  of Stop Solution to each well. Tap gently to mix the stop solution. Avoid splashing.
7. Read the absorbance at 450 nm.

Normal T3 – 77 to 207 ng/dl

## **ESTIMATION OF TOTAL THYROXINE T4 –BY ELISA**

### **PRINCIPLE**

Specific anti-T4 antibodies are coated onto microtitration wells. Test sera are applied.

T4 with Horseradish peroxide enzyme (Conjugate) is added which competes with the released serum T4 for available binding sites on the solid phase.

After incubation, the wells are washed with water to remove any unbound T4 or T4 enzyme conjugate. On addition of the Substrate(TMB), a colour develops only in those wells in which enzyme is present, indicating a lack of serum T4.

The reaction is stopped by the addition of dilute Sulphuric Acid and the absorbance is then measured at 450nm. This test has been calibrated against in house standards. There is no international standard for the test.

#### CONTENTS:

Microtitre Plate			12 * 8 Wells * 1
Breakable wells coated with specific antibodies contained in a reseal able foil bag with a desiccant			
Cal	A	0 ng/ml	1 ml
Reference Standard: Human serum free of T4. Ready to use. (Colourless)			
Cal	B	20 ng/ml	1 ml
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Cal	C	50 ng/ml	1 ml
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Cal	D	100 ng/ml	1 ml
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Cal	E	150 ng/ml	1 ml
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Cal	F	250 ng/ml	1 ml
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Control	Level as stated on vial		1 ml
Known level of T4 diluted in human serum. Ready to use. (Colourless)			
Washbuf		20X	50 ml
Wash Buffer concentrate : Tris based buffer containing			

detergents. (Colourless)				
Conj		11X		1.3 ml
T4 HRP conjugate concentrate: T4 Conjugated to Horseradish Peroxidase. (Colourless)				
DIL		Conj		12 ml
Conjugate Diluent:Phosphate based buffer containing stabilizing proteins. Working Strength(Green)				
Subs		TMB		11 ml
Substrate Solution: 3,3',5,5'TetramethylBenzidine in a citrate buffer.Ready to use. (Colourless)				
Soln	Stop	H2SO4	0.2M	11 ml
Stop Solution: Sulphuric Acid diluted in purified water. Ready to use. (Colourless)				

## REAGENT PREPARATION:

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

**Conjugate:** Dilute the concentrated conjugate using 1 part concentrated conjugate with 10 parts conjugated diluent. eg Add 0.1 ml concentrated conjugate to 1.0 ml of conjugate diluent. This should be done 20 minutes prior to initiation of the assay. Ensure that the diluted conjugate is at room temperature. Do not induce foaming. Use within 24 hours.

Only prepare sufficient working strength conjugate solution to perform the assays required for that day. i.e., 2 strips of 8 wells will require 160 µl of concentrated conjugate diluted in 1.6 ml of conjugate diluent.

**Wash Buffer:** Dilute the concentrated Wash Buffer using 1 part Wash Buffer concentrate with

19 parts distilled water. For every 8-well breakable strip, prepare 25ml of diluted Wash Buffer by adding 1.25ml of concentrated Wash Buffer to 23.75ml of distilled water. Prepare fresh diluted Wash Buffer prior to every assay run. Extra Wash Buffer is supplied to enable priming of automatic washing machines.

The washing procedure is critical to the outcome of this test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

## **ASSAY PROCEDURE**

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assay.
2. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet Provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C .
4. Dispense 25µl of Standards and test serum into the assigned wells.
5. Dispense 100 µl of working strength conjugate into each well.
6. Thoroughly mix for 30 seconds. It is very important to mix completely in this step.
7. Incubate for 60 minutes at room temperature (20°C to 25°C).

8. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectants is contained in the Biohazard container.
9. Hand Washing: Fill the wells with a minimum of 300 µl of wash buffer per well. Flick plate contents into a Biohazard Container. The strike the wells sharply against absorbent paper. Wash the empty wells 5 times.
10. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
11. Machine Washing: Ensure that 300 µl of wash buffer is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
12. Dispense 100 µl Substrate Solution into each well and mix gently for 5 seconds.
13. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C)
14. Stop the reaction by adding 100 µl Stop Solution to each well.
15. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
16. Read the optical density immediately (no later that 10 minutes) using a microplate reader with a 450nm filter.

Normal T4 – 5 to 12 µg/dl

## ESTIMATION OF THYROID STIMULATING HORMONE BY ELISA

### PRINCIPLE

Specific anti-TSH antibodies are coated onto microtitration wells. Test sera are applied. Then goat anti-TSH labelled with Horseradish Peroxidase enzyme (Conjugate) is added. If human TSH is present in sample it will combine with the antibody on the well and the enzyme Conjugate, resulting in the TSH molecule being sandwiched between the solid phase and the enzyme linked antibodies. After incubation, the wells are washed to remove unbound labelled antibodies. On addition of the Substrate (TMB), a colour will develop only in those wells in which the enzyme Conjugate is present, indicating the presence of TSH. The enzyme reaction is stopped by the addition of dilute Hydrochloric acid and the absorbance is then measured at 450nm.

The test has been calibrated to the NIBSC Thyroid Stimulating Hormone 2™ International Reference Preparation 1983 80/558.

### CONTENTS:

Microtitre Plate			12 * 8 Wells * 1
Breakable wells coated with specific antibodies contained in a resealable foil bag with a desiccant.			
Cal	A	0 µ IU / ml	1 ml
Reference Standard: Human serum free of TSH.Lyophilised. (Colourless)			
Cal	B	0.5 µ IU / ml	1 ml
Reference Standard: TSH diluted in human serum. Lyophilised. (Colourless)			
Cal	C	2 µ IU / ml	1 ml
Reference Standard: TSH diluted in human serum. Lyophilised. (Colourless)			
Cal	D	5 µ IU / ml	1 ml
Reference Standard: TSH diluted in human serum. Lyophilised. (Colourless)			
Cal	E	10 µ IU / ml	1 ml
Reference Standard: TSH diluted in human serum.			

Lyophilised. (Colourless)				
Cal	F	25 $\mu$ IU / ml		1 ml
Reference Standard: T4 diluted in human serum.				
Lyophilised. (Colourless)				
Conj				11 ml
TSH HRP Conjugate: TSH conjugated to Horseradish Peroxidase. Ready to use (Red).				
Subs	TMB			11 ml
Substrate Solution: 3,3',5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use (Colourless).				
Soln	Stop	HCl	1M	11 ml
Stop Solution: Hydrochloric acid diluted in purified water. Ready to use (Colourless).				

## **SPECIMEN COLLECTION AND PREPARATION:**

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2° C to 8° C for up to 48 hours prior to testing. If longer storage is required, store at -20° C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

## **ASSAY PROCEDURE:**

- 1) Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assay.



- 2) One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
- 3) Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2° C to 8° C.
- 4) Dispense 100 µl of Standards and test serum into the appropriate wells.
- 5) Dispense 100 µl of Anti-TSH Conjugate into each well. Mix thoroughly for 30 seconds. It is very important to mix completely.
- 6) Incubate for 60 minutes at room temperature (20°C to 25°C).
- 7) Hand Washing: At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
- 8) Fill the wells with a minimum of 300 µl of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 5 times.
- 9) Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
- 10) Machine Washing: Ensure that 300 µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty well 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
- 11) Dispense 100 µl Substrate Solution into each well and mix gently for 5 seconds.

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- 12) Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
  - 13) Stop the reaction by adding 100 µl Stop Solution to each well.
  - 14) Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
  - 15) Read the optical density immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

Normal TSH value – 0.5 to 5 µ units / ml

## **ESTIMATION OF GLUCOSE (GOD-POD METHOD, END POINT) BY AUTOANALYZER**

### **PRINCIPLE**

Glucose + O<sub>2</sub> + H<sub>2</sub>O      Glucose oxidase      Gluconic acid + H<sub>2</sub> O<sub>2</sub>

H<sub>2</sub> O<sub>2</sub> + 4HBA + 4AAP      Peroxidase      Quinoneimine Dye + 2 H<sub>2</sub>O

4AAP : 4 – Aminoantipyrine

4HBA: 4 - Hydroxy benzoic acid

The intensity of the pink colour formed is proportional to the glucose concentration and can be measured photometrically between 500 to 540 nm.

**REAGENT COMPOSITION** (When reconstituted as directed)

**REAGENT 1: Glucose Reagent**

Glucose oxidase	20000 IU/L
Peroxidase	3250 IU/L
4-Aminoantipyrine	0.52 m m ol/L
4-Hydroxybenzoic acid	10 m m ol/L
Phosphate buffer	110 m m ol/L

Also contains non reactive fillers and stabilizers pH 7.0 ± 0.2 at 25 °C.

**REAGENT 2: Glucose Standard**

Glucose Standard	100 mg/dl (5.55 m m ol/L)
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**REAGENT RECONSTITUTION:**

Allow the vial to attain room temperature. Dissolve the contents of each vial using Glucose diluent with special lipid clearing agent. Make up the final volume of 200ml or 500ml depending on the pack size and transfer into a clean dry amber colored bottle.

**ASSAY PARAMETERS:**

Mode	End Point
Wavelength 1(nm)	505
Wavelength 2(nm)	670
Sample Volume (µl)	5 / 10
Reagent Volume(µl)	500 / 1000
Incubation time (min)	15
Incubation Temperature (° C)	37
Normal row (mg/dl)	70

Normal High (mg/dl)	110
Linearity Low (mg/dl)	0
Linearity High (mg/dl)	500
Concentration of Standard (mg/dl)	100
Blank with	Reagent
Absorbance Limit (max)	0.3
Units	Mg/dl

#### ASSAY PROCEDURE:

Pipette into tubes marked	Blank	Standard	Test
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix well and incubate for 15 minutes at 37° C. Read the absorbance of Standard and each sample tube against reagent blank at 505nm (500 – 540nm) or 505 / 670 nm on bichromatic analyzer.

#### CALCULATION:

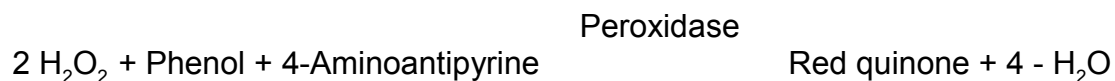
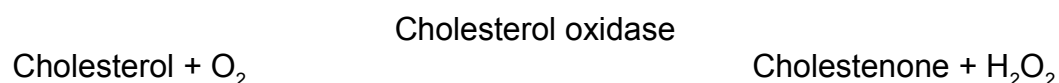
$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Concentration of standard (mg / dl)}$$

Normal Glucose values – 70 to 110 mg/dl (fasting)

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## ESTIMATION OF CHOLESTEROL BY ENZYMATIC METHOD

### PRINCIPLE



The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (Red Quinone) which is measured at 500 nm.

### REAGENTS:

#### Reagent 1 (Enzymes / Chromogen) :

Cholesterol Esterase	≥ 200 U/L
Cholesterol Oxidase	≥ 250 U/L
Peroxidase	≥ 1000 U/L

4 – Aminoantipyrine	0.5 mmol/L
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**Reagent 1A (Buffer):**

Pipes buffer , pH 6.90	50 mmol/L
Phenol	24 mmol/L
Sodium Cholate	0.5 mmol/L

**Standard (Cholesterol 200 mg/dl):**

Cholesterol	2 g/L
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**REAGENT RECONSTITUTION:**

Allow the reagents to attain room temperature. Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1A. Mix by gently swirling.

Write the Reconstitution date in the space provided on the label of bottle 1A. Wait for 5 minutes before using.

**PROCEDURE:**

The Samples and the reconstituted reagent should be brought to room temperature prior to use. The following general system parameters are to be used with this kit.

**General System Parameters**

Reaction Type	:	Endpoint
Reaction Stope	:	Increasing
Wavelength	:	500 nm (492-550)
Flowcell Temp	:	30°C
Incubation	:	5 min at 37°C
Sample Vol	:	10 µL
Reagent Vol	:	1.0 mL
Std. Concentration	:	200 mg/dL

Zero Setting with : Reagent Blank

Set the instrument using above system parameters.

**Dispense into test tubes:**

	Blank	Standard	Test
Reconstituted Reagent	1 mL	1 mL	1 mL
Standard	-	10 µL	-
Sample	-	-	10 µL

Incubate for 5 minutes at 37°C. Mix and read.

Normal Cholesterol Values – 150 to 260 mg/dl

**ESTIMATION OF HDL-CHOLESTEROL BY PHOSPHOTUNGSTATE METHOD**

**PRINCIPLE**

Chylomicrons, VLDL (Very Low Density Lipoproteins) and LDL fractions in serum or plasma are separated from HDL by precipitating with Phosphotungstic Acid and Magnesium Chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant is assayed with enzymatic cholesterol method, using Cholesterol Esterase, Cholesterol Oxidase, Peroxidase and the chromogen 4-Aminoantipyrine/Phenol.

**REAGENTS:**

**Reagent 1 (Enzymes / Chromogen) :**

Cholesterol Esterase	≥ 200 U/L
Cholesterol Oxidase	≥ 250 U/L
Peroxidase	≥ 1000 U/L
4 – Aminoantipyrine	0.5 mmol/L

**Reagent 1A (Buffer):**

Pipes buffer , pH 6.90	50 mmol/L
Phenol	24 mmol/L
Sodium Cholate	0.5 mmol/L

**Reagent 2 (Precipitating Reagent):**

Phosphotungstic Acid	2.4 mmol/L
Magnesium Chloride	39 mmol/L

**Standard (HDL Cholesterol 50 mg/dL):**

Cholesterol	0.5 g/L
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**PROCEDURE:**

The samples, the precipitating reagent 2 and the reconstituted reagent should be brought to room temperature prior to use.

**PRECIPITATION:****Dispense into Centrifuge Tube:**

	Test
Sample	0.20 mL (200 µL)
Precipitating Reagent 2	0.20 mL (200 µL)

Mix well. Centrifuge at 1500g or 3500-4000 rpm for 10 min. Separate the clear supernatant immediately and determine the cholesterol content .

**Dispense into test tubes:**

	Blank	Standard	Test
Reconstituted Reagent	1 mL	1 mL	1 mL
Standard	-	20 µL	-
Supernatant	-	-	20 µL

Incubate for 5 minutes at 37°C. Mix and Read.

Normal HDL Values – 30 to 70 mg/dl

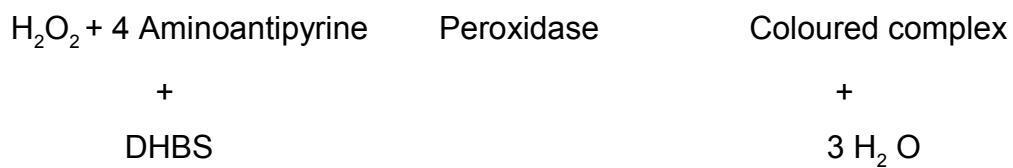
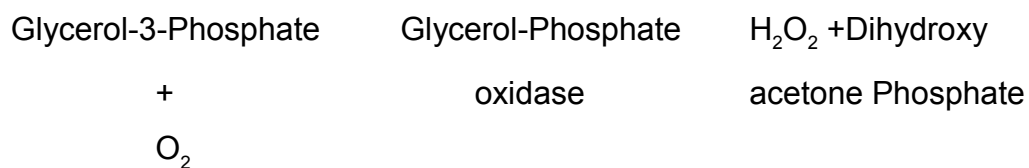
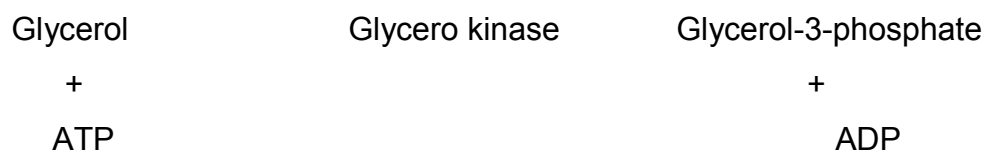
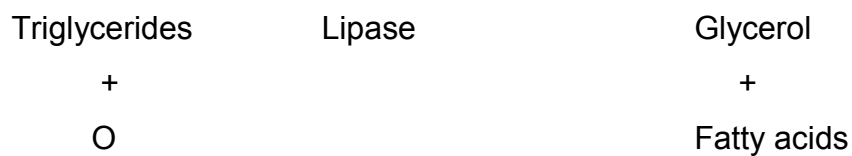


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## ESTIMATION OF TRIGLYCERIDES –GPO METHOD

### PRINCIPLE

Enzymatic determination to Triglycerides by the following reaction



The intensity of coloured complex produced is directly proportional to the concentration of Triglycerides content, which is measured at 520 nm (505-530) nm or with Green filter.

## SPECIMEN COLLECTION:

Fresh, clear, fasting unhemolysed serum is preferred although. Plasma prepared with anticoagulants, such as heparin, citrate may be used.

## GENERAL PROCEDURE:

Type of reaction	:	End Point
Sample Volume	:	10 microliters (0.01 ml)
Working reagent volume	:	1.0 ml
Wavelength	:	520 nm (505-530) nm
Flowcell Temperature	:	30°C
Incubation	:	10 min at 37°C
Light path	:	1.0 cm
Set Zero with	:	Reagent blank

## PROCEDURE FOR 2.5 ML CUVETTE CAPACITY:

	Blank	Standard	Test
Working Reagent (ml)	1.0	1.0	1.0
Standard (ml)	-	0.02	-
Sample (ml)	-	-	0.02
Mix and incubate at 37°C for 10 minutes			
Distilled water (ml)	1.5	1.5	1.5

Mix and read the Absorbance of Standard and test against Reagent blank at 520 nm (505-530nm) or with Green filter, within 30 minutes.

## TEST RESULTS:

Triglycerides concentration =  $\frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 200$   
mg/dl

Normal TGL Level values – 65 to 170 mg/dl

## RESULTS AND DISCUSSION

In our study 60 individuals with IDDM (Male 32, Female 28 in the age group of 10 to 35 years were selected.) Based on the level of anti TPO antibody T3,T4,TSH and lipid profile the biochemical parameters were tabulated, statistically analyzed and evaluated.

In this study 16 persons shows high levels of anti TPO Ab greater than 40 IU. Out of this 10 persons are female and 6 are males (Table-1).

T3 levels were low in 5 persons in anti TPO Ab Positive Individuals (31.3%) (Table 2) (Chart 3).

T4 levels were low in 6 persons (37.5%) (Table-3) (Chart 2).

TSH levels high in 9 persons (56.3%) (Table-4) (Chart 4).

The value of high TSH statistically significant according to the Pearson R formula  $P < 0.001$ .

There is no correlation between duration of IDDM and anti TPO Ab level (Table -7).

There is no statistical correlations between high anti TPO Ab titer and Lipid profile (Table-8).

There is significant correlation between Age and anti TPO Ab level as per the T-Test  $P < 0.049$  (Table-9).

Statistical correlation between BMI and TPO Positive and Negative levels is significant  $P < 0.001$  (Table-10) (Chart 1).

The correlation between weight and TPO Positive and Negative levels is significant in T-Test  $P < 0.001$  (Table-11).

Table-12 shows overall correlation between TPO, Age, Duration of the Disease, Blood – Sugar level and BMI.

There is no significant correlation between Blood-Sugar level and TPO level.

Athero Genic index ( $\log (Tgl / Hdl \text{ in m/mol})$  ). The Athero Genic index in TPO positive individuals in this study were raised in 10 persons (Table-13).

Correlation between T3,T4,TSH and BMI, Weight shows there is significant correlation present.

Prevalance of TPO-Ab with 95% CI is  $p \pm 1.96 \sqrt{0.267 * 0.733 / 60}$

23 to 30. Our value is 26.7 %

## DISCUSSION

Auto immune mechanisms are involved in many cases of Type-1 DM (1). The role of screening for thyroid levels in Type-1 DM is controversial. Substantial prevalence of thyroid abnormalities were noted in patients with Type-1 diabetes mellitus -1 (3),(6) ,(36), (37). The mean age of diagnosis  $19 \pm 2$  in Type 1 diabetes mellitus and  $29 \pm 3$  years. Hypo thyroidism was more common in females than males. Patients who were TPO positive were 17.91 times as likely to develop hypo thyroidism. This statement correlates with our study. There were no differences in BMI and lipid profile (4).

Our results indicate that all Type-1 diabetic individuals should undergo annual screening of serum TSH measurement to detect asymptomatic thyroid dysfunction with positive TPO individuals. In Type-1 diabetes 21.6 % has high level of antibodies to TPO, TG. Thyroid autoimmunity was more common in girls and this predominance was absorbed in all age groups (5),(24),(25). In a study to detect sub-clinically associated AITD. 22 % of patients shows thyreopathy with the assessment of thyroid auto antibodies and TSH.

The screening for auto antibodies in Type-1 diabetic patients will reveal sub-clinical cases of AITD. The sub-clinical thyroid dysfunction have no influence on diabetic control. There is a need for regular follow up of patients with positive auto antibodies to detect further deterioration of other organs(6), (12), (14).

In Type-1 DM Patients with thyroid abnormalities have shown that thyroid volume is increased ultra sonographically. The expression of involvement of the thyroid in an auto immune disorder is not limited to the islet cells (7),(17). In a study on AITD anti-TPO and anti-TG level have increased more in girls than boys. For early detection of auto immune thyroidties in children with measurement of anti TPO and TSH in a yearly intervals after the age of 12 is recommended (8),(9).

A study of thyroid antibodies and TSH level of first degree relatives of Type-1 DM patients and in Anti TPO – Ab positive relatives shows that TSH basal levels were at the upper limit. The TSH level after seven years were increased to  $3.6 \pm 1.9$ . The subjects with

TPO – Ab positivity which shows thyroid auto immunity with TPO – Ab positivity to have be tested regularly on long term basis not only diabetic patients but also in their first degree relatives, in siblings / children (10). AITD are common in adolescents with Type-1 DM.

Seventy patients were performed ultra sonogram of thyroid and TSH measured in 62 persons. TgAb, TPO-Ab levels were estimated in 51 patients. TPO-Ab was found to be increased in 13.7% patients. TgAb slightly increased. US reveals 9 patients having AITD 18% shows hypo echogenicity. There is no correlation between duration of Type-1 diabetes and TPO – Ab (12).

The use of high sensitive immuno metric methods in clinical laboratories to assay anti thyroid anti bodies expanded in recent years. The agreement of qualitative results is close to 97% for anti-TPO (13). In individual with Type-1 DM the measurements of anti TPO-Ab, TSH are the most efficient and cost effective combination of screening test in the early detection of AITD.

The positive predictive value of (anti TPO-Ab and TSH is 90%). In our study there is increased level of anti TPO-Ab and increased level of TSH are noted and low T4 and T3 level indicating sub clinical hypo thyroidism (14),(35),(36).

In a three years follow up of study of Type-1 DM there is prevalence of thyroid dysfunction increased from 5 to 8 %. The prevalence of TPO – Ab is unchanged. All Type-1 DM patients with increased TSH level has US abnormalities while US abnormalities were not always associated with increase in TSH level. Thyroid ultra sound abnormalities was a sensitive but non specific marker of auto immune thyroid diseases. This shows that it is unsuitable for screening purposes.

So we recommend regular annual screening of serum TSH in the follow up of young Type-1 DM (16). Sub clinical phase of thyroid dysfunction in Type-1 diabetes due to auto antibody positivity did not affect control of diabetes while fully manifested hyper , hypo thyroidism impair diabetes control. So there is no influence on diabetic control.

In our study fasting blood glucose level in cases with positive TPO – Ab did not

reveal statistical significance (19,(20). We recommend regular follow up of patients with positive anti TPO-Ab antibody titer to prevent further deterioration of other organs (i.e., thyroid. Hypo thyroidism and its impairment of DM control)

In this study athero genic index log (TGL / HDL ) m/mol of TPO positive and TPO negative individuals shows that there is no statistical significance 10 / 16 (38),(39).

There is inverse correlation between serum TSH level and serum T3,T4 i.e., T3, T4 levels are reduced and TSH is raised . Which indicates sub clinical hypo thyroidism. BMI was calculated and it correlates between TPO – Ab titer. Weight also correlates with the TPO – Ab titer. So sub clinical auto immune thyroid function which is responsible for the weight gain and BMI increase

## **CONCLUSION**

Type-I DM being a chronic auto immune disease which is associates with different auto-anitbodies to thyroid, viz, anit TPO-Ab, anti TGAb and anti thyroid antibodies.

Thyro peroxidase being responsible for iodination of thyrosine moieties, responsible for active thyroid hormone T4 and T3 synthesis, when inhibited by anti TPO Abs

decreases the active T4 ,T3 synthesis resulting in low T4, T3 level and there is a compensatory increase in TSH level, which with increased anti TPO tier and duration leads to deterioration of thyroid function from sub clinical dysfunction to fully manifest clinical hypo thyroidism.

Anti TPO- Abs being the autoimmune parameter measured in Type-1 DM patients. In our study it is characterised by low T4 and T3 levels and increased TSH level.

While in a study it was found anti TPO-Ab with anti TG Abs may present with thyro toxicosis. Similarly anti thyroid Abs in Type-1 DM patients leads to thyro toxicosis.

In this study TPO – Ab were measured. All the cases of TPO – Ab positive individuals show sub clinical hypo thyroidism with elevated level of TSH. So annual screening of TSH is essential to detect and prevent further deterioration into clinical hypo thyroidism and follow up.

Type-1 DM is an auto immune disorder which affects pancreatic beta cell and other endogenous involvement is in not only to pancreas but also to the other organs (Thyroid, Adrenals and non endocrine organs like GI mucosa with Hypo, Hyper thyroidism, Addison's diseases, celiac disease).

There is mean latent period of 10 years between the onset of Type-1 DM and thyroid disease. During which sub-clinical thyroid dysfunction turns into clinical hypo function. So annual screening of TSH level is recommended in anti TPO positive individuals with Type-1 DM.

AITD also has influence on DM control because hypo or hyper thyroidism will affect the diabetic control while sub-clinical hypo thyroidism has no effect on diabetic control. In anti TPO positive Type-1 DM patients estimation of TSH level raise should be done annually, in order to detect hypo function of thyroid , even in sub clinical itself so as to maintain Euglycemic status.

In reference 20, based on study of 237 diabetic children the authors proposed the following screening protocol.



- 1) Thyroid dysfunction coexisted with Type-1 DM in 5.5% of their cohort but not with Type-2 DM.
- 2) Screening for fT4 and TSH levels identifies asymptomatic children.
- 3) Thyroid dysfunction was strongly associated with thyroid auto-antibodies.
- 4) Thyroid function should be assessed annually in diabetic children.

Based on their study the author recommend that

- 1) fT4 and TSH level should be measured at diagnosis and annually thereafter.
- 2) Thyroid auto – antibodies should be measured at diagnosis of Type-1 DM and should be repeated if TSH level exceeded the reference range.

While the other studies suggest , Type-1 DM young patients with anti TPO Abs develop clinical thyroid disease with a mean latent interval of 10 years from the onset of Type-1 DM, during which sub clinical thyroid dysfunction occurs and it should be suffice to measure TSH level annually in anti TPO positive Type-1 DM patients with which we concur.

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